A fully defined and scalable 3D culture system for human pluripotent stem cell expansion and differentiation.

Journal:	Proc Natl Acad Sci U S A
Publication Year:	2013
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PubMed link:	24248365
Funding Grants:	Interdisciplinary Training in Stem Cell Biology, Engineering and Medicine, The Berkeley Human Embryonic Stem Cell Shared Research Laboratory, Engineering Defined and Scaleable Systems for Dopaminergic Neuron Differentiation of hPSCs, Human Stem Cell Training at UC Berkeley and Childrens Hospital of Oakland

Public Summary:

Human pluripotent stem cells (hPSCs), including human embryonic stem cells and induced pluripotent stem cells, are promising for numerous biomedical applications, such as cell replacement therapies, tissue and whole-organ engineering, and high-throughput pharmacology and toxicology screening. Each of these applications requires large numbers of cells of high quality; however, the scalable expansion and differentiation of hPSCs, especially for clinical utilization, remains a challenge. We report a simple, defined, efficient, scalable, and good manufacturing practice-compatible 3D culture system for hPSC expansion and differentiation. It employs a thermoresponsive hydrogel that combines easy manipulation and completely defined conditions, free of any human- or animal-derived factors, and entailing only recombinant protein factors. Under an optimized protocol, the 3D system enables long-term, serial expansion of multiple hPSCs lines with a high expansion rate, high cell density, and purity, all of which offer considerable advantages relative to current approaches. Moreover, the system enabled directed differentiation of hPSCs into multiple lineages, including dopaminergic neuron progenitors with a high yield and purity. This versatile system may be useful at numerous scales, from basic biological investigation to clinical development.

Scientific Abstract:

Human pluripotent stem cells (hPSCs), including human embryonic stem cells and induced pluripotent stem cells, are promising for numerous biomedical applications, such as cell replacement therapies, tissue and whole-organ engineering, and high-throughput pharmacology and toxicology screening. Each of these applications requires large numbers of cells of high quality; however, the scalable expansion and differentiation of hPSCs, especially for clinical utilization, remains a challenge. We report a simple, defined, efficient, scalable, and good manufacturing practice-compatible 3D culture system for hPSC expansion and differentiation. It employs a thermoresponsive hydrogel that combines easy manipulation and completely defined conditions, free of any human- or animal-derived factors, and entailing only recombinant protein factors. Under an optimized protocol, the 3D system enables long-term, serial expansion of multiple hPSCs lines with a high expansion rate (approximately 20-fold per 5-d passage, for a 1072-fold expansion over 280 d), yield (approximately 2.0×10^7 cells per mL of hydrogel), and purity (approximately 95% Oct4+), even with single-cell inoculation, all of which offer considerable advantages relative to current approaches. Moreover, the system enabled 3D directed differentiation of hPSCs into multiple lineages, including dopaminergic neuron progenitors with a yield of approximately 8×10^7 dopaminergic progenitors per mL of hydrogel and approximately 80-fold expansion by the end of a 15-d derivation. This versatile system may be useful at numerous scales, from basic biological investigation to clinical development.

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